

Megazyme

MEASUREMENT OF
XYLANASE
IN ANIMAL FEEDS

using

AZO-WAX
SOLUTION

FDAZWAX 11/99



INTRODUCTION:

Arabinoxylan is the major endosperm cell-wall polysaccharide of wheat and rye and is found in significant proportions in most cereal solutions and slurries of high viscosity, and in animal nutrition it reduces the rate of nutrient absorption from the gut.

endo- β -D-Xylanase (xylanase) is added to feeds to catalyse depolymerisation of this polysaccharide. It can be demonstrated that *endo*-cleavage by xylanase of just one bond per thousand in the arabinoxylan backbone can significantly remove viscosity properties.

Of the carbohydrase enzymes used as feed supplements, one of the most difficult to measure has been xylanase. These problems are attributed to several factors, including the low levels of enzyme added to the feed, inactivation of enzyme during pelleting, binding of the enzyme to feed components and inhibition by soluble feed components.

The only biochemical methods which are sufficiently sensitive, specific and robust to measure xylanase in feeds are viscometric assays and those employing dyed xylan or arabinoxylan polysaccharides. Viscometric assays are tedious, whereas assays employing dyed xylan substrates are rapid, reproducible and simple to perform. We recommend the use of either Xylazyme AX tablets or Azo-Wheat Arabinoxylan (AZO-WAX). Xylazyme AX based assays are about 5-fold more sensitive than assays employing AZO-WAX, however, this latter substrate does have sufficient sensitivity in most applications, and results are slightly more reproducible than with Xylazyme AX.

It is generally accepted that xylanase enzymes which are best suited to feed applications have optimal activity at pH 6.0. Consequently, these enzymes are generally assayed at this pH in 0.1M sodium phosphate buffer. However, we have shown that sodium phosphate buffer extracts only a small proportion (< 20%) of the amount of enzyme added to the feed (in "recovery" experiments). Consequently, we have evaluated a wide range of alternative extractants and extraction conditions and our final recommendations for the extraction and assay of xylanase in feeds is detailed below.

Of all the conditions and extractants evaluated, the best and most consistent results for feeds containing *Trichoderma* sp. xylanases have been obtained using 0.1M acetic acid or 0.1M sodium acetate buffer (pH 4.6) at room temperature. Optimal extraction of *Humicola* sp. xylanases is achieved with a buffer containing MES buffer salt (0.1M, pH 6.0; Sigma M-8250) and sodium dodecyl sulphate (SDS; 1% w/v).

KIT COMPONENTS:

Kits containing the required reagents to measure xylanase in animal feeds are available from Megazyme. These kits contain:

1. Azo-Wheat arabinoxylan
2. *Trichoderma sp.* Control xylanase (6800 milli-Units/ml) solution in 50% glycerol

EXTRACTION BUFFERS: (not enclosed)

(A) Sodium Acetate Buffer (0.1M, pH 4.6)

Glacial acetic acid (6.0 g, 1.05 g/ml) is added to 800 ml of distilled water. This solution is adjusted to pH 4.6 by the addition of 5M (20g/100 ml) sodium hydroxide solution. The volume is then adjusted to 1 litre. Store at room temperature

(B) MES/SDS Buffer

[MES (0.1M, pH 6.0) plus SDS (1%)]

MES free acid (Sigma M-8250; 19.5g is added to 900ml of distilled water and dissolved. The pH is adjusted to 6.0 with 1M sodium hydroxide, and SDS (lauryl sulphate, Na salt; Sigma L-4509; 10g) is added and dissolved. The volume is adjusted to 1 litre.

EQUIPMENT (RECOMMENDED):

1. Glass test tubes (round bottomed; 16 x 100mm and 16 x 120mm)
2. Micro-pipettors eg: Gilson Pipetman 500 microlitre and 100 microlitre
3. Positive displacement pipettor eg: Eppendorf Multipette® - with 5.0mL Combitip® (to dispense 0.2ml aliquots of xylanase control in 50% glycerol)
4. Adjustable volume dispenser set at 5.0mL (to dispense Trizma Base solution)
5. Top-pan balance correct to 0.01g
6. Spectrophotometer set at 590nm
7. Vortex mixer (we recommend the Thermolyne Maxi-Mix II)
8. Whatman No. 1 (9cm) filter circles and filter funnels.

WITHOUT GUARANTEE

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EXTRACTION AND ASSAY OF XYLANASE IN FEED SAMPLES

Trichoderma sp. Xylanases:

EXTRACTION:

1. A feed sample (approximately 100 grams) is milled to pass a 0.5mm screen and mixed thoroughly.
2. Samples of the above feed ($0.5 \pm 0.01\text{g}$ in quadruplicate) are weighed into glass test-tubes (16 x 120mm).
3. Each sample is treated with 5ml of 0.1M sodium acetate buffer (pH 4.7) and stirred on a vortex mixer. To two of these tubes, water (0.2ml) is added with stirring, and to the other two tubes is added control *Trichoderma* sp. xylanase (0.2ml, 1360 mUnits) with vigorous and immediate stirring on a vortex mixer.
4. The slurries are left at room temperature with occasional stirring on a vortex mixer over the following 20 minutes.
5. Tubes are centrifuged (3,000 rpm, 10 min) in a bench centrifuge and the supernatant is used directly in assays. Assays should be initiated within 30 minutes of obtaining these extracts to minimise loss of enzyme activity..

ASSAY:

1. Accurately transfer 0.5ml aliquots of supernatant solutions (in duplicate) to glass test-tubes (16 x 100 mm) **at room temperature**.
2. Add 0.5 ml of AZO-WAX to each tube and stir the tube vigorously. Immediately place the tube in a water bath set at $50^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ and incubated for exactly 30 min.
3. After exactly 15 or 30 min (depending on the level of enzyme activity) , add 2.5 ml of Industrial Methylated Spirits (IMS) and stir the tube vigorously on a vortex mixer. Store the tube at **room temperature** for 5 minutes.

This treatment terminates the reaction and precipitates non-depolymerised dyed substrate.

4. Centrifuge the tubes at 3,000 rpm for 10 min.
5. Measure the absorbance of the supernatant solutions at 590 nm against a reaction blank.

The Reaction Blank is prepared by adding 2.5 ml of IMS to a mixture of 0.5 ml of AZO-WAX and 0.5 ml of 0.1 M sodium acetate buffer (pH 4.6). The slurry is stirred and stored at room temperature for 5 minutes before centrifugation (3,000 rpm, 10 min).

A single reaction blank is required for each feed sample.

CALCULATION OF ACTIVITY:

The level of xylanase in the flour sample is calculated as follows:

$$\text{Activity in feed sample (0.5g)} = \text{Added activity} \times \frac{\text{SA}}{\text{TA} - \text{SA}}$$

WHERE:

Activity added = the amount of xylanase added to the feed slurry at the time of assay eg: 1360 mUnits in the control xylanase solution (0.2ml).

SA = the reaction absorbance obtained for extracts of the feed to which no control xylanase was added.

TA = the total absorbance ie: the absorbance of extracts of the sample to which the control xylanase was added.

EXAMPLE CALCULATION:

Sample	ABS/30 min. incubation
1. Feed A	0.000
2. Feed A containing <i>Trichoderma</i> sp. xylanase (SA)	0.502
3. SA + 1360mU xylanase (in the assay) (TA)	0.908

$$\text{Activity in 0.5g of feed A} = \text{Added activity} \times \frac{\text{SA}}{\text{TA} - \text{SA}}$$

WHERE:

- SA = absorbance of **extract of sample A**
[assayed by the standard format (eg: 0.502)]
- TA = **total absorbance**; ie: the absorbance of extracts of **sample A** to which the additional xylanase (0.2ml; 1360 mUnits) was added (eg: Abs = 0.908).

THUS:

Activity in feed

(Units/0.5g);

$$= 1360/1000 \text{ Units} \times 0.502/(0.908 - 0.502)$$

$$= 1.360 \times 0.502/(0.908 - 0.502) = \mathbf{1.682 \text{ Units.}}$$

Units/Kg (ie. K Units/ton);

$$= 1.682 \times 2000 = \mathbf{3363}$$

REFERENCE:

McCleary, B.V. "Problems in the measurement of β -xylanase, β -glucanase and α -amylase in feed enzymes and animal feeds". In "**Proceedings of Second European Symposium on Feed Enzymes**" (W. van Hartingsveldt, M. Hessing, J.P. van der Lugt and W.A.C. Somers Eds.), Noordwijkerhout, Netherlands, 25-27 October, 1995.



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